

Supplemental Data

Nitration with peroxynitrite treatment yields a mix of nitrated and unnitrated peptides. Analysis of the TOF revealed a triply charged peak at 814.04, which corresponds to F295-R314. The mass of this peptide is 71Da greater than would be expected and is the result of a propionamide modification to C308, which was confirmed by analysis of the y ions. Tyrosine nitration of this peptide would result in a 15Da shift in mass, a peak at 829, which is shown in Figure S1.

To confirm that m/z 829 and m/z 814 were the same peptide we compared the y and b ions. Figure S2 shows the product ion spectra for these two m/z values. We can see by looking at these ions that the sequence is identical on the amino and carboxy terminal ends. The b₂₋₉ ions and the y₁₋₆ ions of each CID spectrum match. Because we are not able to sequence through the tyrosine we can not see the 45Da shift in the b or y ions, and therefore can only say that the evidence strongly suggests that this peptide is nitrated. The nitration of this peptide would be consistent with mutant studies that we have performed.

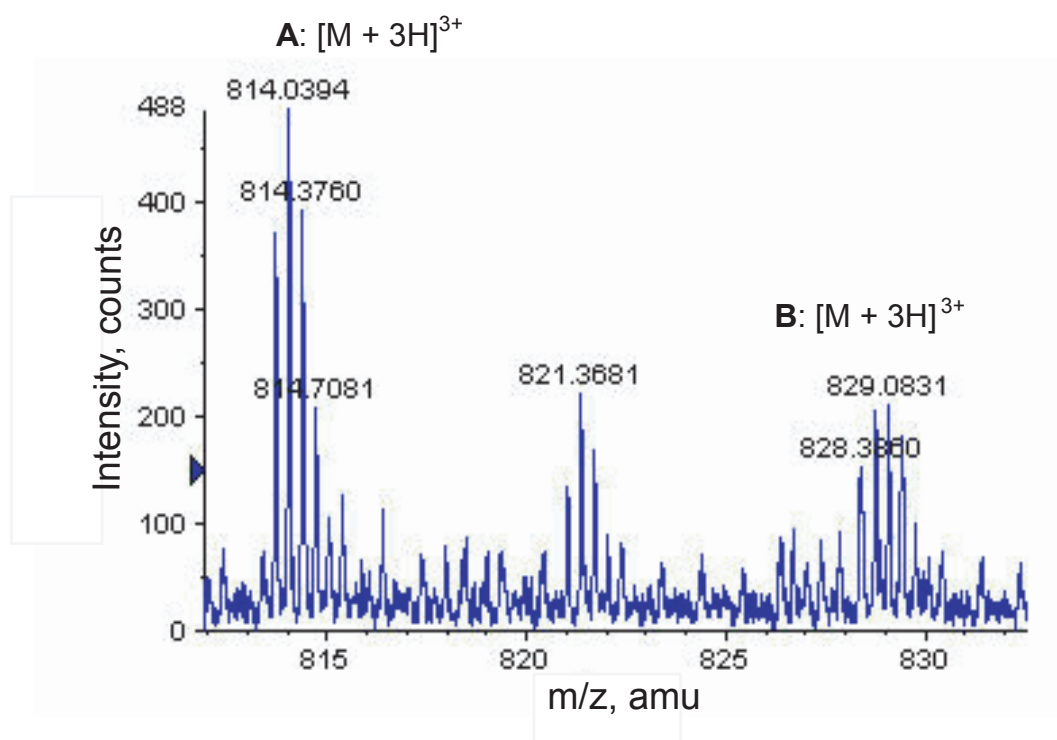


Figure S1. Highlighted region of the TOF mass spectrum of a tryptic digest of peroxynitrite-treated IkB α . TOF MS reveals the presence of two peptides at $[M + 3H]^{3+} = 814$ and $[M + 3H]^{3+} = 829$ that differ by 15 Da.

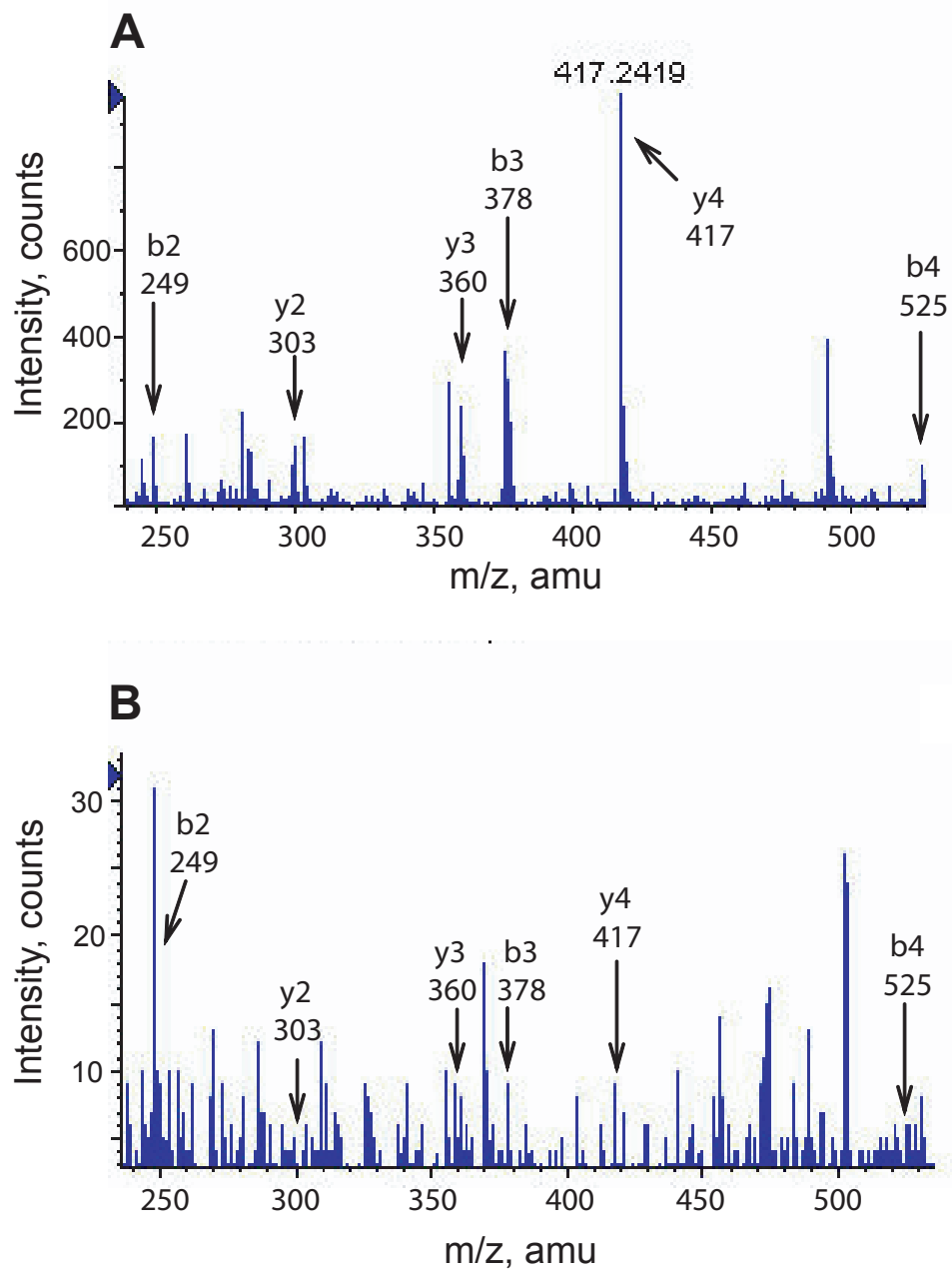


Figure S2. The MS/MS spectrum for (A) $[M + 3H]^{3+} = 814$ and (B) $[M + 3H]^{3+} = 829$. Selected y and b ions are shown.

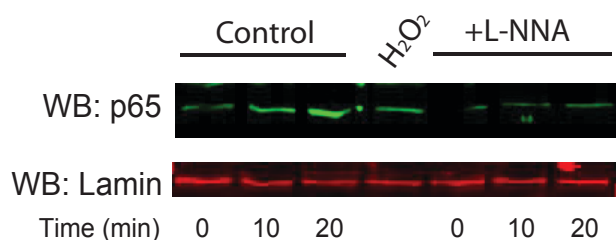


Figure S3. NOS activity modulates NF- κ B nuclear translocation.

Cells were irradiated at 5 Gy and the nuclear fraction of cell lysates was analyzed by immunoblotting for p65. Cells were incubated for 4 h with 100 nM L-NNA prior to IR. At the indicated time cells were harvested and the nuclear fraction was isolated as described (3). As a positive control 1 hour incubation with 1 mM H_2O_2 was used. Equal loading was verified by blotting with anti-lamin (bottom panel).

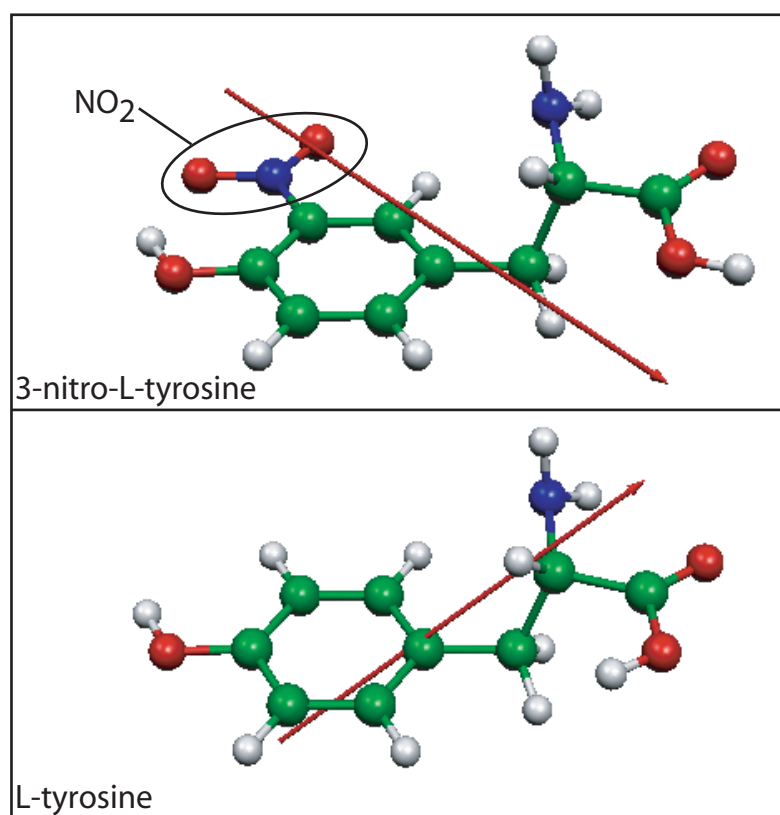


Figure S4. The dipole moment vectors for tyrosine and nitro-tyrosine.

The dipole moment vectors are represented by red arrows; the length of the vector corresponds to the normalized scalar component of the dipole moment to allow for direct comparison. The dipole moment is 3.63 Debye for tyrosine and 5.78 Debye for nitro-tyrosine.

Table S1. Mass spectroscopy results for I κ B α protein: observed m/z values and their corresponding A.A. sequence.

Amino acids	Peptide Sequence	Theoretical mass	Calculated mass ^a	Ion Charge	Modifications
54-61	LEPQEVPR	966.51	966.54	MH ₂ ²⁺	None
88-95	ALTMEVIR	1269.69	1269.73	MH ₂ ²⁺	None
265-275	IQQQLGQLTLE	931.52	931.57	MH ₂ ²⁺	None
295-314	FTEFTEDELPYDDCVFGGQR	2367.0	2439.1	MH ₃ ³⁺	Propionamide ^b
295-314	FTEFTEDELPYDDCVFGGQR	2367.0	2484.1	MH ₃ ³⁺	Propionamide + NO ₂ ^{b,c}
246-260	VTYQGYSPLYQLTWGR	1817.87	1818.9	MH ₂ ²⁺	None

a - Calculated masses were determined from the observed m/z values of doubly or triply charged species;

b - Mass is consistent with Propionamide modification of cysteine;

c - Mass is consistent with tyrosine nitration.